Photodegradation of Riboflavin to Lumichrome in Milk Exposed to Sunlight

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ABSTRACT

A simple, qualitative procedure for detecting the photodegradation of riboflavin in milk exposed to sunlight is reported. Lumichrome (7,8-dimethylalloxazine) has been identified by mass spectrometry as the major product of the photodegradation.

INTRODUCTION

The effect of light on the organoleptic and nutritive properties of milk has been investigated extensively (5). Noticeably lacking in several of those investigations is an objective procedure for detecting milk samples that have undergone photochemical reactions. This report summarizes our initial studies to develop such an analytical procedure and establishes lumichrome as a major photodegradation product of riboflavin in milk exposed to light.

EXPERIMENTAL

Pasteurized skim milk was prepared from raw herd milk obtained from a local dairy farm. Unless specifically stated, all operations of sample preparation and experimental procedure were in the absence of direct lighting.

Thin-layer Chromatographic (TLC) Detection of the Photodegradation of Riboflavin

Four 15-ml samples of pasteurized skim milk in 15 × 1.2 cm Pyrex[1], screw cap, culture tubes were exposed to diffuse sunlight for 0, 5, 10, and 20 min. The samples were passed (flow rate: 1.5 ml/min) through individual columns of Amberlite XAD-4 prepared in the following manner: 1 g of resin in methanol was added to a 50 × .6 cm glass buret which was fitted with a Teflon stopcock and plugged with glass wool. The resin was washed consecutively with 25 ml each of methanol, acetone, and distilled water at a flow rate of 2.0 ml/min, with care that solvent remained above the resin at all times. A plug of glass wool was added to the top of the resin prior to adding the skim milk samples. Following passage of the samples through the column, the resin was washed free of sample with 25 ml of distilled water and eluted with 8 ml of methanol. The effluent was evaporated to dryness in a 4.5 × 1.2 cm screw cap vial. The residue was dissolved in .25 ml of methanol with gentle heating and 2.0 μl were spotted on a 10 × 20 cm silica gel G TLC plate (.25 mm thickness). The TLC plate was developed 6 cm with the upper layer of a mixture of n-butanol:acetic acid:water (4:1:5), air dried, and observed under ultraviolet light for fluorescing compounds.

Isolation and Characterization of Lumichrome

Two hundred milliliters of pasteurized skim milk were exposed to diffuse sunlight for 50 min in a 60 × 2.0 cm glass column and then passed through 5 g of Amberlite XAD-4 (flow rate: 2.5 ml/min) in a 35 × 1.0 cm glass column fitted with a glass stopcock and plugged with glass wool. (The column was prepared as described above except that 50 ml of methanol, acetone, and distilled water were used in washing the resin.) The resin was washed free of skim milk with 75 ml of distilled water and eluted with 30 ml of methanol. The methanol effluent was evaporated to dryness under a stream of nitrogen. A total of 1.5 ml of distilled water (at increments of .75, .5, and .25 ml) was added to the residue, warmed to approximately 50 C, and spotted across seven 20 × 20 cm silica gel G TLC plates (.5 mm thickness). The TLC plates were developed 12 cm with n-butanol:acetic acid:water (4:1:1), dried overnight at

Received January 24, 1977.

[1] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

45 C in a vacuum oven, and the fluorescent area corresponding chromatographically to lumichrome was removed from the plates. The adsorbent was packed in a 12 × 1.7 cm glass column plugged with glass wool, washed with 10 ml of methylene chloride, and the fluorescent material was eluted with 10 ml of a 10% solution of methanol in methylene chloride. The effluent was evaporated to dryness under a stream of nitrogen. The residue was taken up in 1.5 ml of distilled water and extracted with 4 to 5 ml portions of methylene chloride. The combined methylene chloride extracts were dried with sodium sulfate and evaporated to dryness under a stream of nitrogen. The residue was dissolved in .75 ml of methanol, spotted across five 20 × 20 cm silica gel G TLC plates (.5 mm thickness), and developed 15 cm with benzene:methanol:acetic acid:butanone-2 (70:20:5:5). The fluorescent area corresponding to lumichrome was recovered from the adsorbent, extracted into methylene chloride and freed of solvent according to the procedure described above.

Authentic lumichrome was purchased from Pfaltz and Bauer, Inc., Stamford, CT, and purified further by TLC employing the n-butanol:acetic acid:water (4:1:1) solvent system. Recovery of lumichrome from the adsorbent was accomplished as described above.

Ultraviolet-visible (UV-Vis) Spectrometry

Ultraviolet-Vis spectral analyses were obtained in methanol utilizing the Carey Model 14 Spectrometer and 1-cm quartz cuvettes.

Mass Spectrometry

Mass spectra were obtained on a Hitachi-MRU-6E Mass Spectrometer, interfaced to the INCOS 2300 Data System, under the following conditions: electron energy—70 ev; trap current—55 μA; accelerating voltage—1.8 KV; source temperature—230 C, and probe temperature programmed from 70 to 100 C.

RESULTS AND DISCUSSION

Brewington and Schwartz (1) reported that riboflavin was removed from milk by adsorption on a neutral resin and recovered, near quantitatively, by desorption with polar organic solvents. Employing Amberlite XAD-4 in conjunction with TLC, we observed a decrease in the riboflavin content with a concomitant increase in the concentration of a developed fluorescent spot from milks exposed to sunlight for increasing lengths of time (Fig. 1). Since no changes in concentration of other fluorescent spots (one major and several minor) on the TLC plate were perceivable, we concluded that the emergence and increasing concentration of the aforementioned fluorescent spot was related to the photodegradation of riboflavin. Tentative identification of the fluorescent compound as lumichrome was based on: 1) TLC mobility in three solvent systems; 2) characteristic fluorescence on the TLC plate in the presence and absence of acetic acid (7); and 3) the UV-Vis spectra (Fig. 2).

Conclusive evidence for the identification of lumichrome was obtained by comparison of the mass spectra of the isolated compound with authentic lumichrome (Fig. 3) in which the molecular ion (m/e 242) is the most intense peak. The major fragmentation pattern of lumichrome results in the expulsion of HNCO

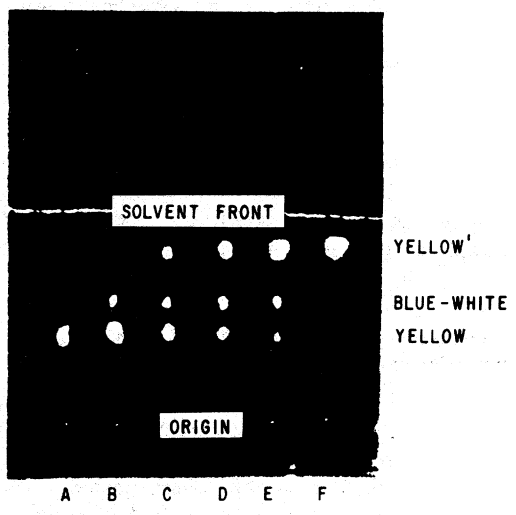

FIG. 1. Tracing of thin-layer chromatogram under ultraviolet light, demonstrating the photodegradation of riboflavin in milk exposed to sunlight. A—Authentic riboflavin; B—Control skim milk; C—Skim milk exposed 5 min; D—Skim milk exposed 10 min; E—Skim milk exposed 20 min; F—Authentic lumichrome. Weak fluorescent spots not included in tracing. [1] Fluorescent color of lumichrome changes to blue-white on evaporation of acetic acid.

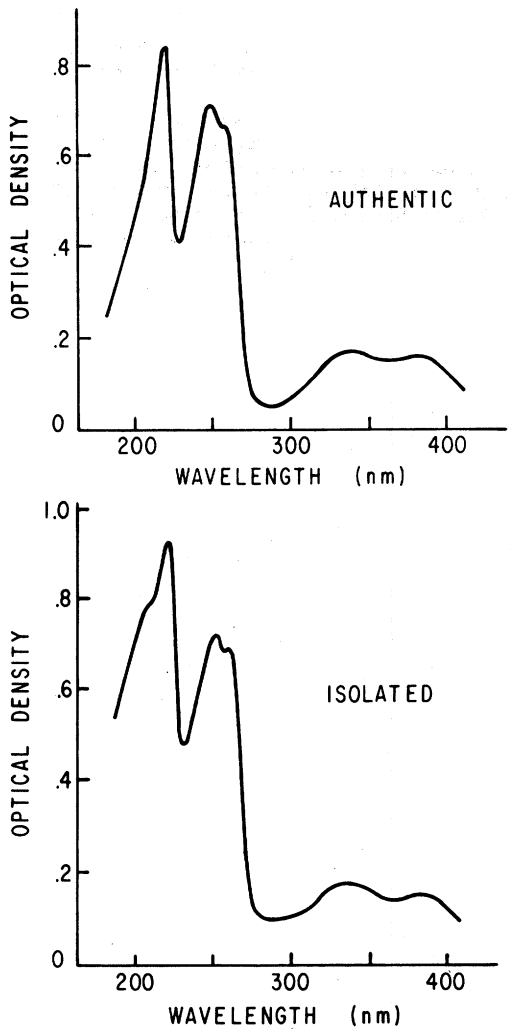

FIG. 2. Comparison of UV-Vis spectra of lumichrome isolated from skim milk exposed to sunlight and the authentic compound.

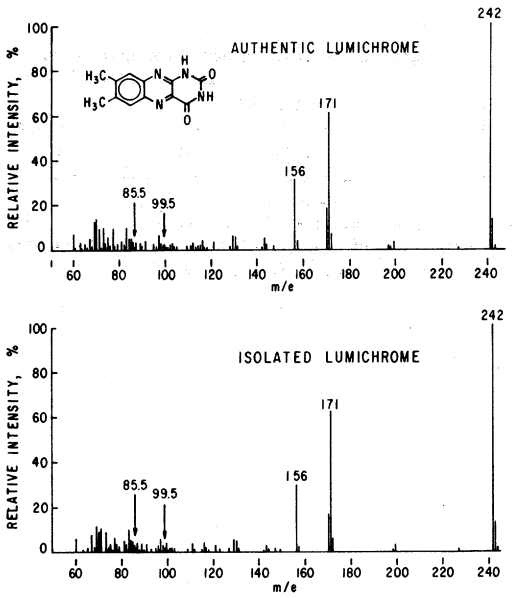

FIG. 3. Comparison of mass spectra of lumichrome isolated from skim milk exposed to sunlight and the authentic compound.

from the tautomeric pyrimidine ring (m/e 199) followed by the elimination of CO (m/e 171) and CH_3 (m/e 156) (2). Metastable peaks at 163.6 (m/e 242 → 199), 146.9 (m/e 199 → 171), 142.3 (m/e 171 → 156), and 121.3 (m/e 171 → 144), as well as doubly charged ions at m/e 85.5 and m/e 99.5 present in the isolated and authentic sample conclusively confirm the identity of the compound.

The products formed by the photodegradation of riboflavin in model systems depend on the wavelength of light, length of exposure, the presence or absence of both oxygen and electron donors, and the pH of the medium (3, 4, 6). In addition to riboflavin and lumichrome, lumiflavin, a fluorescent isoalloxazine formed by the photodegradation of riboflavin in model systems could be isolated by the procedure employed here. These procedures also may be capable of isolating other fluorescent isoalloxazines that have been reported as photodegradation products of riboflavin. Hence, the absence of increased quantities of fluorescent compounds other than lumichrome in milks exposed to sunlight leads us to conclude that lumichrome is the major photodegradation product of riboflavin in milk.

ACKNOWLEDGMENT

We are grateful to Vincent P. Flanagan, Lipid Nutrition Laboratory, Beltsville, MD, for the mass spectral analyses.

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